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GERMINATION PATTERN AND CAPACITY FOR REPEATED STROMA FORMATION IN *CLAVICEPS PURPUREA*

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(With 4 Text-figures)

Ergot populations have been studied with respect to germination, stroma formation and ascospore discharge. Discharge occurs over a longer period than has previously been supposed. Periodic removal of clavae from germinated sclerotia results in a larger number of stromata being formed than if ergots are undisturbed. The physiological significance of this is discussed.

The epidemiology of ergot caused by *Claviceps purpurea* (Fr.) Tul. poses many interesting problems and among these are considerations of some factors affecting initial infection of the host by ascospores. Sclerotia, having fallen to the ground in the autumn, become wholly or partially buried during the winter, are stimulated to germinate by low winter soil temperatures and subsequently do so when soil temperatures become warmer in the spring. Stromata are raised above the soil surface and ascospores are ejected into the atmosphere to infect the grass host.

For heavy initial infection, discharge of the ascospores should synchronize with early anthesis of the particular host attacked and primary infections are said to be more successful when the flowers have just opened, presumably because germ tubes grow better in the absence of interference from a deposit of pollen. Fresh infections may take place for about 15 days after flowering has begun, the infection being heavier the longer the flowers are open (Butler & Jones, 1961). If ascospores are released before the flowering of the host they must remain in the atmosphere for long enough to allow infection when flowering subsequently occurs.

It appears that in nature sclerotia from *Lolium perenne* begin to germinate in early June and have finished by the third week of the same month (Jenkinson, 1958). It has been stated that a period of about a week elapses from the first signs of germination to maturation of the stromata and that spore discharge may occur for the relatively short period of about 14 days after this. Initial infection takes place in the early part of July, with later infections in August and September attributed to the conidial state of the fungus (Jenkinson, 1958). In the laboratory, sclerotia from rye stimulated by a period of chilling will germinate over a period of 14 days (Kirchhoff, 1929) while those from *Phalaris arundinacea* do so over a period of 27–30 days (Cooke & Mitchell, 1966).

Petch (1937) has pointed out that synchronization of sclerotium germination and ascospore discharge with host flowering does not inevitably take place. Ergots from *Phragmites communis*, for example, germinate in June long before this grass flowers in August.

Although the literature on *Claviceps purpurea* is large there is little information available as to over how long a period a population of sclerotia could be expected to continue to germinate or as to the pattern of ascospore release during this time. The criteria used in assessing germination have not been made clear and no distinction has been attempted between initial germination and subsequent stroma formation. It is also commonly supposed that a sclerotium is capable of producing only one crop of stromata. However, it has been observed that if clavae are removed from germinated ergots of rye then some sclerotia subsequently germinate again (Kirchhoff, 1929). Similarly if stromata of *C. paspali* are removed from their stipes then new stromata will form at the cut ends (Grasso, 1950). Should sclerotia be capable of a series of germinations then this would effectively spread the infectivity of a population over a much longer period than would otherwise be expected and so increase the chance of establishing primary infections.

In the investigation to be described the possibility of repeated sclerotium germination was explored and a study made of the pattern of stroma production and ascospore discharge during the germination period.

GERMINATION PATTERN AND ASCOSPORE DISCHARGE

In all experiments the sclerotia used were from a single population gathered from a pure stand of *Phalaris arundinacea* in Lathkill Dale, Derbyshire, in September 1965. Work on these ergots was carried out from November 1965 to May 1966. They were air-dried and stored at room temperature before use.

In any experiment batches of at least 40 sclerotia were used. Sclerotia were allowed to imbibe water for 2–4 h, were placed on damp filter paper in closed dishes and incubated at 5 °C for 8 weeks to induce germination. At the end of this period they were placed on fresh, damp filter paper in closed dishes and allowed to germinate at room temperature. An ergot was considered germinated as soon as the first clava had emerged from the sclerotial tissue and the number of germinations was usually scored at daily intervals. At the same time the number of stromata arising from each sclerotium was also counted. Stroma production was expressed in terms of the number arising at any time from 100 sclerotia. In order to obtain information as to the pattern of ascospore liberation during the germination period three batches of ten germinating sclerotia were fastened to the lids of three 6 cm Petri dishes by means of 'Araldite' resin. In the bottom of each dish was placed 2 ml of distilled water and at 24 h intervals the bottoms were exchanged for new ones containing fresh water. This process was repeated for the duration of the experiment. Stromata discharged their ascospores into the water and these were counted using a haemacytometer.

A typical pattern for germination, clava emergence and ascospore discharge is shown in Fig. 1. Germination began 15 days after removal of sclerotia from the chilling regime and reached a maximum 16 days later. Clavae continued to emerge for a considerable period after the population had reached maximum germination and the number of stromata per 100 sclerotia reached a maximum of 300 56 days after the onset of germination. New stromata were often formed below old and shrivelled ones on the same stipe or at the stipe bases.

Ascospore discharge reached a level detectable by the method used 14 days after the onset of germination, rose to a maximum in excess of 16000 spores per sclerotium between 11 and 14 days later and fell to an undetectable level by the 38th day after the first germination had taken place. The total period of detectable ascospore discharge was 24 days. Examination of stromata indicated that perithecia were mature 7 days after emergence from sclerotial tissue and probably began to discharge ascospores at this point. First-formed stromata would therefore liberate spores 7 days after the onset of germination but these would be too few (less than 50 spores per sclerotium) to be detected by the method used. It is probable that spores were still being discharged at levels below this for some time at the end of the detectable period. The total period of ascospore discharge from a population is therefore probably in excess of 50 days.

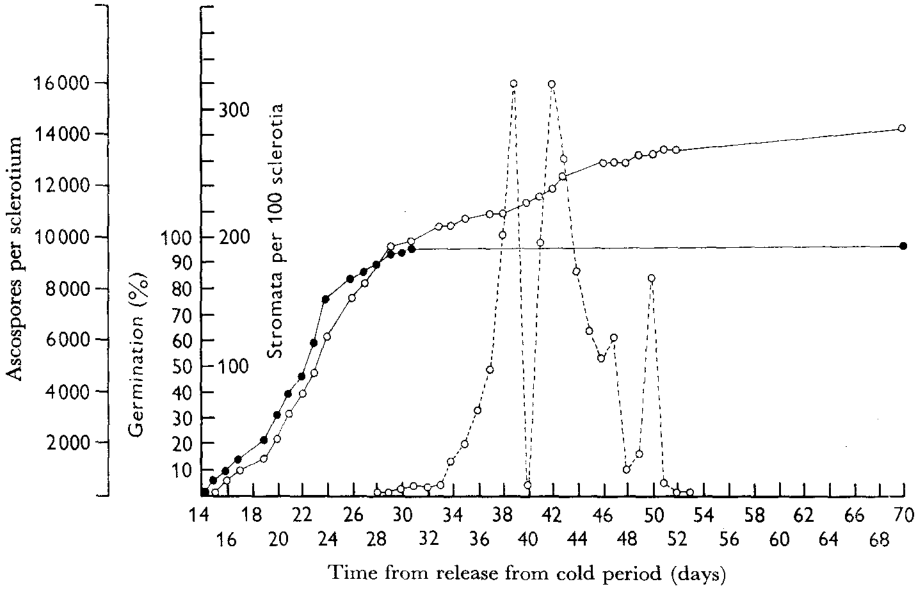

Fig. 1. Percentage germination, number of stromata per 100 sclerotia and number of ascospores discharged from each sclerotium for a population of ergots during a 70-day period subsequent to its removal from a chilling temperature. —●—, Percentage germination; —○—, stromata per 100 sclerotia; - -○- -, ascospores per sclerotium.

REPEATED STROMA FORMATION

Air-dry sclerotia were weighed and divided into classes (Table 1). In any experiment batches of 50 sclerotia of each class were used except in the case of class 5 where usually only about 20 sclerotia were available. Ergots were treated as described above and germination scored at daily intervals. After being allowed to reach maximum germination the stalks of the stromata arising from each ergot were cut off flush with the sclerotium surface. Sclerotia were then left for a time comparable with the first germination period, germination was scored and any stromata removed as before. This process was repeated until little or no germination subsequently occurred after removal of stromata. At the end of each germination period the average number of stromata produced by each germinated sclerotium in each weight class was noted. A sclerotium was considered 'germinated' as soon as it was apparent that a new stroma was arising from it. The period for any sample of sclerotia to reach maximum percentage germination was 18–20 days from the emergence of the first clava. After removal of stromata at the end of this time sclerotia produced a further crop and this process could be repeated up to three times at 21- to 25-day intervals.

Table 1. *Weight classes of sclerotia from* Phalaris arundinacea

	Class				
	1	2	3	4	5
Weight (mg)	1–3	3–5	5–7	7–9	>9

New stromata were formed in three ways. First, there could be regeneration of the cut ends of the stipes, but as these did not increase in length the stromata were borne close to the sclerotium surface. Secondly, new clavae were produced from the old stromatal cushions at the old stipe bases and stromata developed at the tips of these new clavae. Stipes and stromata appeared normal except that they were frequently unpigmented. Finally, clavae were produced from new stromatal cushions at previously ungerminated sites under the sclerotium surface and again these gave rise to unpigmented but otherwise normal stromata. These three methods of stroma formation could all occur simultaneously on a single sclerotium. Only a minority of new stromata were produced by clava regeneration and about equal numbers by the other two methods.

Results for percentage germination with time from classes 1 and 2 were very similar and these were pooled as were those from classes 3 and 4. The number of sclerotia producing new stromata during the second and subsequent germination periods varied with the weight class (Fig. 2). The smaller weight classes showed a much reduced capacity for producing new stromata after the first germination while this was less pronounced in heavier sclerotia. As well as the number of sclerotia giving rise to stromata decreasing, the rate at which stromata were produced also fell after the first germination. By the end of the fourth germination period stroma production was very low in all weight classes. At the end of this time some sclerotia had been producing stromata for 90 days.

The average number of stromata arising from each germinated sclerotium correspondingly fell with successive germination periods, although large sclerotia of classes 3, 4 and 5 were producing more stromata during their second germination than sclerotia from classes 1 and 2 were during their first (Fig. 3). At the time that stromata were removed it was estimated that 60–80 % of them contained ripe perithecia and 10 % of stromata had released their ascospores and were shrivelling.

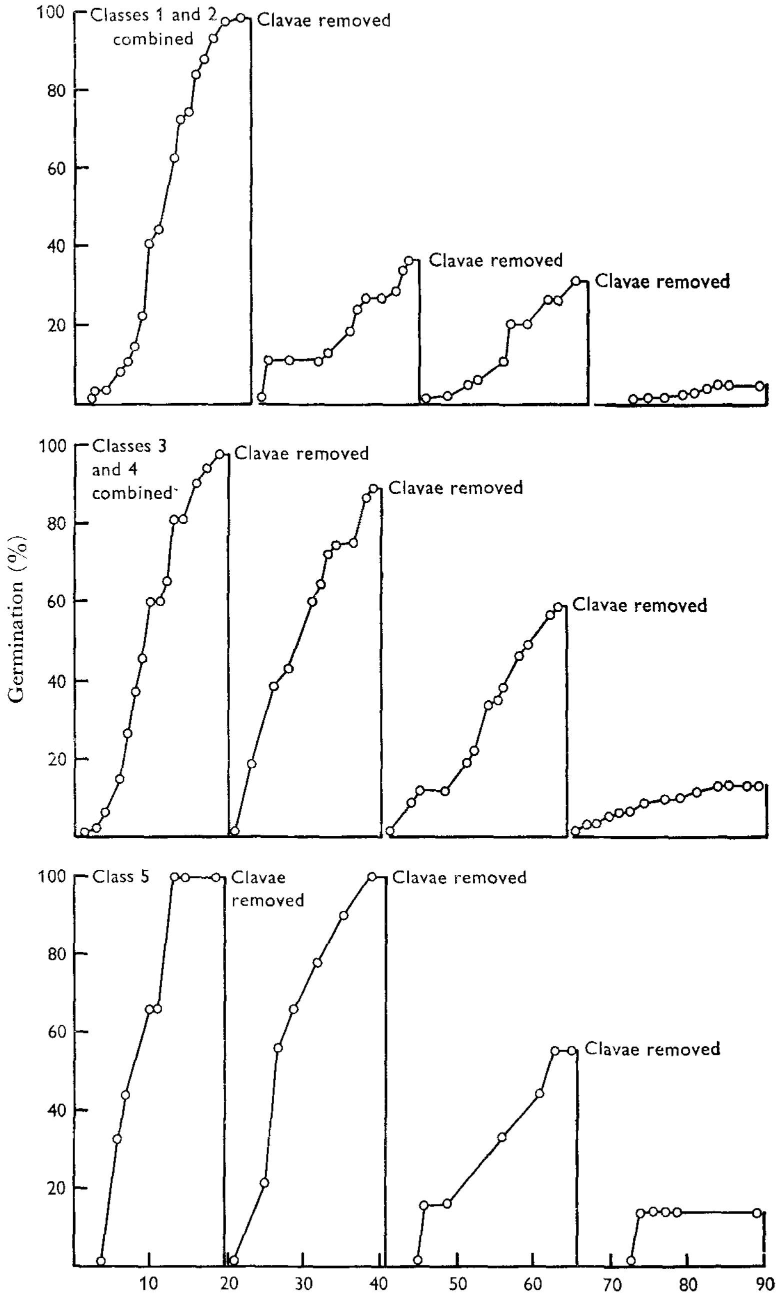

Fig. 2. Percentage germination of different weight classes of sclerotia after periodic removal of clavae.

Batches of class 1–4 sclerotia were allowed to germinate undisturbed as controls. Percentage germination and the number of stromata arising from each ergot were scored at daily intervals (Fig. 4). Clavae continued to emerge for a considerable period after maximum germination had been reached and the number of stromata per 100 sclerotia was at a maximum 20–32 days after the onset of germination. At this time there were averages of 130, 197, 347 and 348 stromata per 100 sclerotia in classes 1, 2, 3 and 4 respectively.

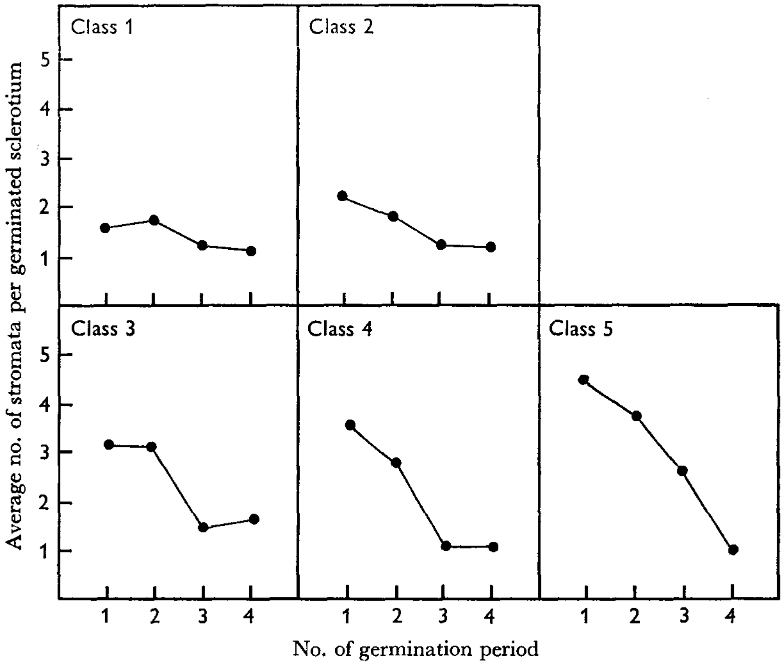

Fig. 3. Average number of stromata arising from germinated sclerotia during successive germination periods after removal of clavae.

POTENTIAL FOR STROMA PRODUCTION

Infectivity of an ergot population depends upon the number of stromata which it is capable of producing and this is related to the size of the constituent sclerotia (Cooke & Mitchell, 1966). The *maximum potential* for stroma production of a population may be defined as the maximum total number of stromata which could possibly arise from 100 sclerotia taken at random from that population. It is a reflexion of both the viability of the constituent sclerotia and of the ability of those which do germinate to produce stromata. The *realized potential* of a population at any particular moment in time is equal to the total number of stromata arising from 100 ergots taken at random at that particular time.

Considering the experiments using weight classes of sclerotia it is apparent that removing stromata before ascospore discharge is complete induces ergots to produce more stromata over a period than does leaving them undisturbed. Where stromata were removed, realized potential at the end of each germination period equals percentage sclerotia germinated × the average number of stromata per germinated sclerotium (Table 2).

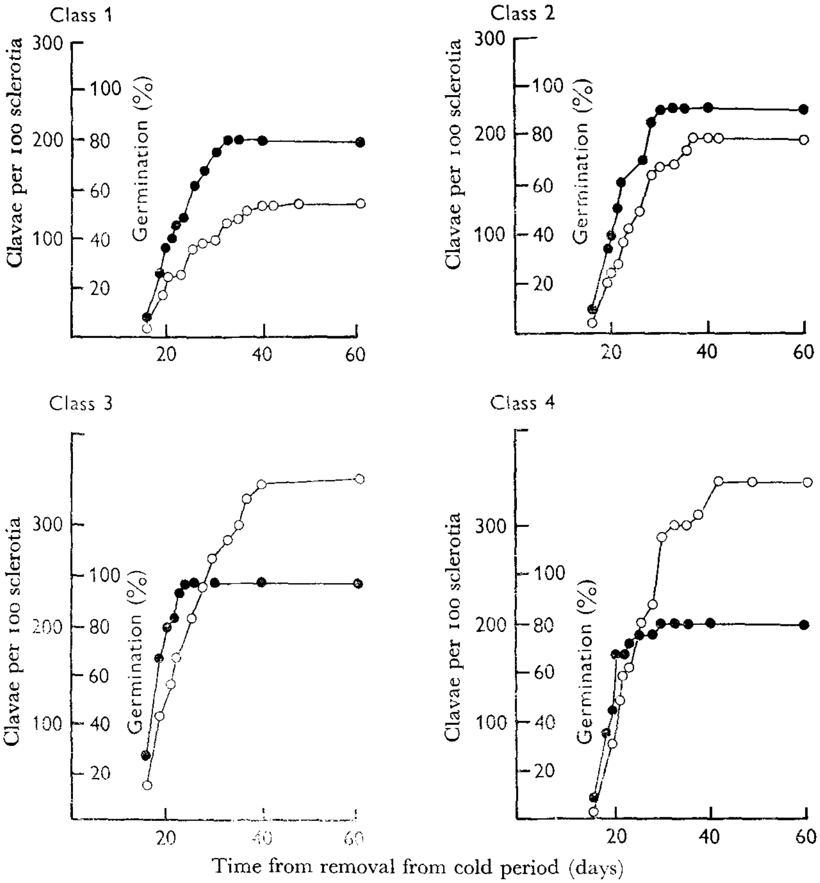

Fig. 4. Percentage germination and number of stromata per 100 sclerotia for different weight classes of ergots allowed to germinate undisturbed after a chilling period. ●, Percentage germination; ○, stromata per 100 sclerotia.

Table 2. *Realized potential for stroma production by sclerotia at the end of successive germination periods*

		Weight class				
		1	2	3	4	5
Germination Period	1	150	210	310	380	450
	2	54	82	295	247	400
	3	38	47	92	64	143
	4	2	12	19	13	14
Total realized potential over 90 days		244	351	716	704	1007
Total realized potential, undisturbed sclerotia		130	197	347	348	—

DISCUSSION

In a population of sclerotia when percentage germination has reached a maximum, new stromata can continue to be formed up to 56 days from the onset of germination. Ascospore discharge continues for a much longer time than has formerly been supposed and spores may be released over a period in excess of 50 days. The apparent biological problem of the release of spores to the atmosphere in synchronization with host anthesis is thus reduced.

Undisturbed sclerotia never realize their maximum potential for stroma formation and exhaust all their food reserves during limited stroma production and ascospore release. Removal of stromata from sclerotia before the majority of the former have liberated their ascospores results in the formation of a new crop of stromata. This process can be repeated several times.

There appear to be a number of potential sites within a sclerotium from which stromatal cushions may be formed and these are all stimulated into activity by a period of chilling. The larger the sclerotium the more sites it is capable of supporting. Some sites will develop quicker than others and once one site has passed a certain stage of development the formation of clavae from adjacent sites is in some way inhibited. If left undisturbed stromata mature and release ascospores, in so doing utilizing the available energy reserves in the sclerotium. If clavae are removed before the majority of stromata have released their ascospores then other cushion primordia are released from a state of inactivity and produce stromata.

In small sclerotia proportionally more of their reserves or stromatal primordia are used up during the first germination so that in subsequent germinations the increment of realized potential increases very little. With larger sclerotia considerable reserves and primordia remain unexploited after the first germination so that in subsequent periods realized potential may increase greatly. Stroma formation can be repeated with gradually diminishing intensity until all energy reserves are utilized or alternatively all primordia are used up.

A mechanism seems to exist therefore whereby only about half the potential sites for stroma production are in fact used during the germination process (Table 2) and energy is directed solely to these sites for ascospore production and release. This effects economical use of available reserves in increasing the chances of those stromata that are formed of producing and liberating large numbers of ascospores. Such a mechanism was hinted at after casual observations by Wilson (1876), who remarked: 'Certain points in the mass of mycelium composing the ergot are more vital than others, and at these points growth takes place, and probably withdraws vegetative force from the points immediately around it.'

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